A study on loading multiple epitopes with a single peptide

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Abstract

Epitopes, the basic functional units of antigens, hold great significance in the field of immunology. However, the structure and composition of epitopes and their interactions with antibodies remain unclear, which limits in-depth studies on epitopes and the development of subunit vaccines. In a previous study on the localization of anti-influenza HA monoclonal antibodies (mAbs), three strains with different characteristics reacted with the same peptide. In this study, by conventional immunological assays, computer homology modeling, and molecular docking simulations, we found that 1) the peptide could bind to three strains of mAbs with different reaction characteristics utilizing different combinations of immunodominant

groups. 2) By computer molecular docking and simulation methods, the immunodominant groups on the two peptides could be combined into a multi-epitope peptide bound to six strains of mAbs. We established a method for multi-epitope peptide recombination from these immunodominant groups. 3) The immune effect of the recombinant multi-epitope peptide was better than that of a single peptide. Our findings facilitate the understanding of the composition of antigen epitopes and provide a theoretical and experimental basis for developing polyvalent vaccines and understanding immune responses at the molecular level.

KEYWORDS

epitope, immunodominant group, monoclonal antibody, molecular simulation, multi-epitope peptide

1 INTRODUCTION

Immune responses are initiated by antigen recognition, and its basic unit is the antigen epitope.[1-3] According to the traditional description of "epitope," it can bind to antibodies or activate immune cells and is a fixed and special structure with a certain composition.[4,5] However, in reality, the failure of vaccine development against human immunodeficiency virus (HIV),[6] the decrease in vaccine titer caused by mutations in COVID-19,[7] and adverse reactions caused by influenza vaccine vaccination suggest insufficient knowledge about antigen epitopes. It is possible that these structures are not fixed.[8,9] Thus, improving the immune efficacy of the vaccine and eliminating the epitopes that cause adverse reactions necessitates further studies. Further investigations on the composition and structure of epitopes are required to optimize the epitopes to improve vaccine effectiveness and safety.

A typical peptide epitope comprises 5–6 amino acids; a typical saccharide epitope comprises 5–7 monosaccharides, and a single nucleic acid epitope typically comprises 6–8 nucleotides. Nevertheless, it is unclear whether a peptide comprising 5 or 6 amino acids is represented as a single or multiple epitope.[10,11] Amino acids of epitopes significantly affect antibody binding, among which several key residues play

the most important roles; such amino acids are termed immunodominant groups. Over the last few decades, numerous epitopes have been comprehensively analyzed, yielding a wide array of immunodominant groups.[12-15]

Our research group synthesized mAbs against the influenza virus hemagglutinin (HA) in its early stages. In a localization study of these mAbs,[16] 2–4 strains of mAbs with different reaction characteristics were found to bind to the same influenza virus peptide. When comparing the immunodominant groups, only 1–2 amino acids differed, suggesting that the epitope may be a combination of immunodominant groups.

We investigated if the immunogenicity of an antigen can be improved if the immunodominant groups of several peptides are recombined into one peptide to form a multi-epitope peptide. Considering the above question, this study was conducted to elucidate the molecular basis of immune reactions and provide a theoretical and experimental basis for developing polyvalent vaccines.

MATERIALS AND METHODS

Detailed descriptions of the experiments are provided in Supporting Information SI: Sections *S1 and S2*.

2 METHODS

2.1 Identifying the reaction characteristics of six mAbs with different antigens

An indirect enzyme-linked immunosorbent assay (ELISA) was performed. In brief, 96-well plates were coated with influenza virus HA antigens of 5 μg/mL overnight at 4 °C. The plates were washed, and antigens were blocked. Purified mAb (3 mg/mL) was serially diluted two-fold and added to the plates; SP2/0 was used as a negative control, and mAb against the HA-conserved area of influenza A virus was a positive control. HRP peroxidase-conjugated sheep anti-mouse secondary antibody (1:2500 dilution) was added. A TMB-H_2O_2 chromogenic solution was used as the substrate. The reaction was terminated with $2MH_2SO_4$, and the absorbance at 450 nm was measured. The ratio of each test sample (OD450) to the negative control (OD450) was calculated. Samples with ratios ≥2.5 were considered positive reactions *(SI Methods 2.1).*

2.2 Localization of mAb recognition epitopes

We previously mapped the epitopes recognized by influenza virus HA mAbs, including the six mAbs in this study.[16] The detailed process of epitope localization was shown in *SI Methods 2.2*. The HA crystal structure of the H1N1 influenza virus was obtained from the Protein Databank (PDB). The protein sequence of 3LZG was used as a reference to locate the distribution regions of the mAb recognition epitopes on the HA crystal using the PyMOL software.

2.3 Identifying immunodominant groups for the six mAbs

For the localized peptides, each amino acid in the sequence was individually replaced with an alanine residue, resulting in the synthesis of a collection of alanine-replacement peptides (Table S1). A 96-well ELISA plate was coated with 100 μL of 5 μg/mL H1N1 influenza virus HA antigen. The six mAbs (3 μg/mL) were incubated with the localized peptide (100 μg/mL) and the family of alanine replacement peptides (100 μg/mL) at 37 °C for 1 h. The mixtures were added to the pre-coated ELISA plates and incubated at 37 °C for 1 h. After washing, HRP-conjugated goat anti-mouse secondary antibody (1:2500 dilution) was added and incubated at 37 °C for 1 h. After washing, a TMB coloring solution was added as a substrate for HRP. The OD450 values were measured using an ELISA reader. The inhibition rate (IR) was calculated as follows: (control group-experimental group)/control group. Correlations between the HA antigen and binding peptides and alanine-replacement peptides of mAb were defined as follows: IR $\leqslant$ 0.4, no correlation; 0.4 $\leqslant$ IR $\leqslant$ 0.8, correlation, and IR $\geqslant$ 0.8, strong correlation.[17]

2.4 Preparation and characterization of mAbs against LP-9 and WE-6

The peptide carboxyl end was activated with an EDC activator (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide) and coupled with the carrier protein, bovine serum albumin (BSA) or keyhole chimecyanin (KLH). Cross-linking of the peptides to the carriers was outsourced to Shanghai Qiangyao Biological Company.

The peptides LP-9 and WE-6 of carboxyl-terminally cross-linked KLH were the immunogens. Preparation and characterization of mAbs are described in *SI Methods 2.4.1 and 2.4.2*

2.5 Analysis of structures of the six mAbs and their interaction with peptides

2.5.1 Cloning, sequencing, and homology modeling of the light and heavy chain variable region of the six mAbs

mRNA was extracted from six mAbs hybridoma cells and synthesized into cDNA for gene cloning following a previously described method.[17] Cloning, sequencing, and homology modeling of light and heavy chain variable regions of six mAbs were performed *(SI methods 2.5.1).* [18]

2.5.2 Interaction between six mAbs and peptides

The 3D structures of peptides LP-9 and WE-6 were constructed using the UCSF Chimera software.[19] Using the Rosetta software,[20] six mAbs were docked with LP-9 and WE-6. During the flexible docking process, the receptor protein was positioned at the center of the globular system, and the peptide was evenly distributed in the spherical polypeptide-binding pocket *(SI methods 2.5.2)*.

2.6 Recombination of multiple epitope peptides

Immunodominant groups of six mAbs were split, merged, and recombined. Recombinant multi-epitope peptides were constructed by homology modeling and optimized by molecular dynamics simulations. The variable region modeling structure of the six mAbs and the recombinant multi-epitope peptides that could bind well to the six mAbs were screened based on indicators, including surface accessibility, hydrophilicity, and affinity *(SI methods 2.6)*.

2.7 Screening and validation of the recombinant multi-epitope peptides

In the competitive ELISA, 96-well plates were coated with H1N1- HA antigen of 5 μg /mL at 4 °C overnight. The plates were washed thrice with phosphate-buffered saline, and the antigen was blocked with 1% BSA (mg/mL) at 37 °C for 2 h.

Recombinant multi-epitope peptides and mAbs were mixed and incubated at 37°C for 1 h followed by addition in plates containing the HA antigens. ELISA was performed according to the methods described in *SI Methods 2.2*. Recombinant multi-epitope peptides were screened based on their IR range. All peptides were tested thrice on different days, and the average of three separate runs was the final titer value.

The identified multi-epitope peptides or control antigens were used as coating antigens (10 μg/mL), and their reactivity with six mAbs was assessed by indirect ELISA as described in *SI methods 2.1*. Purified mAbs and control antibodies (3 mg/mL) were diluted 10-fold for ELISA. OD (positive)/OD (negative) ≥ 2.5 was determined as a reactivity cut-off value.

2.8 Preparation, identification, and neutralization assay for immune serum against recombinant multi-epitope peptide

The recombinant multi-epitope peptides were conjugated to BSA and KLH carrier proteins using EDC activators for immunization. Preparation, characterization, and neutralization assays of the immune serum against multi-epitope peptides are described in *SI methods 2.8*.[21]

3 RESULTS

Detailed descriptions of the results in Supporting Information SI: Sections *S3*.

3.1 mAbs with different reaction characteristics recognize the same peptide

We previously mapped the epitopes recognized by H1N1 influenza virus HA mAbs, including six mAbs: H1-5, H1-16, H1-81, A1-10, H1-74, and H1-84.[16] IR of the six mAbs binding to peptides 1 and 2 (WE-6 and LP-9) was > 0.8; however, IR of the six mAbs binding to the other eight peptides (peptides 3– 9) was < 0.4. H1-5, H1-16, and H1-81 could bind to peptide 1, WSYIVE (93aa-98aa), and A1-10, H1-74, and H1-84 could bind to peptide 2, LVLWGIHHP (191aa-199aa). The distribution of these two peptides on the HA trimer crystal structure is marked in red and blue, located in the β sheets of the HA head region, as shown in Figure S1. *(SI results 3.1)*

In this study, the six mAbs were incubated with various influenza virus antigens, including the 2009 influenza A (H1N1) virus lysis vaccine, influenza viruses H1N1 (501 and PR8), H3N2, H5N1, H7N2, and H7N9, and evaluated by ELISA. Appropriate negative and positive controls were used, and the reaction results are shown in Table 1. Although A1-10, H1-74, and H1-84 recognized the same peptide, LP-9, they exhibited inconsistent response characteristics with 501, H5N1, and H7N2. H1-5, H1-16, and H1-81 simultaneously recognized WE-6 but their reactions with PR8 and H7N2 differed, suggesting that they may recognize different epitopes.

3.2 mAbs bindings to the same peptide recognize different immunodominant groups

Amino acids in LP-9 and WE-6 were replaced with alanine. As shown in Table S1. ELISA was performed to identify the immunodominant groups of the six mAbs mapped to different alanine replacement peptides. IR was calculated by measuring the OD450 values. As shown in Figure 1A and 1B, WE-6 and LP-9 were the positive controls. When the alanine replacement peptide could not block the binding of the mAbs to HA, the IR was < 0.4, suggesting that the alanine replacement site was an immunodominant group for mAb binding. Conversely, when the alanine replacement peptide blocked the binding of the mAbs to HA, the IR was > 0.8, suggesting that the alanine replacement site was not an immunodominant group for mAb binding.

The three mAbs bound to the same peptide and recognized three different epitopes, although immunodominant groups overlapped (Figure 1C).

3.3 Peptides LP-9 and WE-6 may form multiple epitopes

Following immunization with the two peptides, mAbs against LP-9 and WE-6 were prepared using conventional hybridoma cell fusion and screening techniques. These mAbs reacted with immunogens but showed different reaction characteristics from those of other antigens. Among the anti-LP-9 mAbs, only LP-9-6 reacted with the H1N1 and H3N2 influenza virus antigens. LP-9 peptide may contain at least two epitopes: one same as the epitope on the H1N1 and H3N2 viruses, and one which was

different (Table S2, *SI results 3.3*). Although seven of the anti-WE-6 mAbs reacted with different subtypes of influenza virus antigens, four (WE-6-11, WE-6-12, WE-6-15, and WE-6-16) showed similar response characteristics. The other three mAbs had different response characteristics (Table 2). Thus, the WE-6 peptide comprises multiple epitopes.

3.4 Different sequences of variable regions on the six mAbs

Molecular biology methods were used to obtain the amino acid sequences of the light- and heavy-chain variable regions (V_L and V_H) of the six mAbs. The analysis software was used to analyze the skeleton regions (FRs) and complementarity-determining regions (CDRs) of VL and VH, as shown in Figure S2A and S2B (*SI results 3.4*). A comparison of the amino acid sequences of the CDRs and FRs of VL and VH for the six mAbs revealed significantly changed amino acids in FR and CDR. The three CDRs of the V_L and V_H together constitute the antigen-binding site of the mAb, which recognizes and binds to the antigen. The six mAbs were derived from different cell clones, and although three mAbs reacted with peptide LP-9 and three mAbs reacted with peptide WE-6, they recognized different immunodominant groups, that is, different epitopes.

3.5 Binding interactions between six mAbs and two peptides

The crystal structures of the six mAbs remain unresolved, and the 3D structure of each mAb was constructed by homology modeling. Crystal protein structures sharing >77% sequence similarity with H1-5, H1-16, H1-81, A1-10, H1-74, and H1-84 mAbs were selected as templates for homology modeling (Table S3).

The binding modes of the six mAbs with LP-9 and WE-6 were predicted by molecular docking simulations using the Rosetta software. The docking conformation with the lowest binding energy for each system was extracted for further analysis. As shown in Figure 2, a highly effective binding interface featuring a robust polar and hydrophobic binding network was formed between LP-9 and multiple amino acids in A1-10, H1-74, and H1-84 mAbs. The binding energies among the three mAbs were

relatively similar, ranging from -20.7– -22.8 kcal/mol (Tables S4A), suggesting a substantial binding affinity between the peptide and the antibody. In contrast to LP-9, peptide WE-6 formed a binding interface with mAbs H1-5, H1-16, and H1-81. However, each antibody's peptide and binding pockets showed a good polarity and hydrophobic binding match, with energies ranging from -14.63 to -15.40 kcal/mol (Tables S4B). As show in Table S4, each immunodominant group on the WE-6 and LP-9 peptides could interact with multiple amino acid residues in the variable region of different mAbs, and different mAbs could bind to different immunodominant groups with varying combinations of their variable region amino acid residues.

3.6 Construction of recombinant multi-epitope peptides and their binding affinities with six mAbs

Based on *in silico* predictions, we recombined the residues that bind to the six mAbs and obtained 61 recombinant multi-epitope peptides, each containing six mAb-binding epitopes. The free energies of binding between the artificial recombinant peptides and the six mAbs were obtained using molecular dynamics simulations. Six recombinant multi-epitope peptides (XH-001: ILLWVLSHL, XH-002: LLIWVLSHL, XH-003: ILIWVLSHI, XH-004: ILIWVLSHV, XH-005: ILIWVISHL and XH-006: ILIWVLSHL) with a binding affinity of <-8.0 Kcal/mol to each mAbs were obtained (Figure S3). The binding energies of the six recombinant peptides and six mAbs were all below 8.0 Kcal/mol, indicating that the recombinant peptides showed good binding with mAbs (Table 3).

3.7. Screening and validation of the six recombinant multi-epitope peptides

As shown in Table 3, the six recombinant peptides exhibited good binding activity with the six mAbs. Thus, six recombinant multi-epitope peptides were synthesized. The reactivity of recombinant multi-epitope peptides to the six mAbs was assessed by competitive ELISA and indirect ELISA. In the competitive ELISA experiments, an IR $\leq$ 0.4, no correlation; 0.4 $\leq$ IR $\leq$ 0.8, correlation, and IR $\geq$ 0.8, strong correlation. As shown in Figure 3A, only IR for XH-001 and XH-006 for

binding to the six mAbs were all above 0.8, indicating that the XH-001 and XH-006 recombinant multi-epitope peptides were recognized by the six mAbs.

Indirect ELISA was performed to observe reactivity between the six mAbs and the XH-001 and XH-006 recombinant multi-epitope peptides. XH-001 and XH-006 peptides were synthesized and labeled with BSA. The six mAbs (3 mg/mL) were serially diluted 10-fold, and the ratio of the test sample to the negative control, if greater than 2.5, was considered a positive result. The results (Figure 3B) indicated that the identified XH-001 bound to six mAbs, distinct from the binding of the control peptide and control antibody SP2/0. The XH-001 peptide combined well with six mAbs following the dilution of the XH-001 peptide and fixing the antibody concentration (Figure 3C).

3.8 Recombinant multi-epitope peptide: XH-001, IRLWVLSHL, which generated antibodies that reacted with influenza virus antigen

BALB/c mice (6–8 weeks old) were immunized with XH-001 labeled with the carrier protein KLH to prepare the anti-recombinant multi-epitope peptide XH-001 serum. The serum was tested for antibodies against LP-9, WE-6, and H1N1 influenza viruses to determine whether the multi-epitope peptides contained the antigen epitopes of LP-9, WE-6, and the influenza virus HA antigen, with the control peptide serving as the antigenic control. When anti-XH-001 peptide serum was diluted to 0.3 – 0.003 μg/mL, it reacted with the LP-9, WE-6, and H1N1 antigens (Figure S4 and Table 4). XH-001 recombinant multi-epitope peptide carried epitopes of LP-9 and WE-6, and the epitopes on the HA protein of the influenza virus were well replicated to the recombinant multi-epitope peptides by the six strains of anti-influenza; these epitopes were well displayed when immunizing animals.

The neutralization activity of XH-001 immunized serum against A/California/07/2009(H1N1) influenza virus was evaluated. The results are shown in Figure 4. XH-001 immunized serum could neutralize A/California/07/2009(H1N1) influenza virus at 640-fold dilution to a concentration of 4.69 μg/mL. Compared with XH-001 immunized serum, anti-LP-9 antibody could neutralize

A/California/07/2009(H1N1) influenza virus at 160-fold dilution with a concentration of 18.75 μg/mL, anti-WE-6 antibody could neutralize A/California/07/2009(H1N1) influenza virus at 40-fold dilution with a concentration of 75 μg/mL; simultaneously, XH-001 immunized serum showed 50% neutralization activity against A/California/07/2009(H1N1) influenza virus at 1280-fold dilution with a concentration of 2.34 μg/mL (Figure 4A-C). The protective effects of XH-001 on MDCK cells are shown in Figure 4E. Compared with the negative control (Figure 4E, E1) and control antibodies (Figure 4E, E3), XH-001 immunized serum exerted a significant protective effect on MDCK cells (Figure 4E, E2).

4. DISCUSSION

The epitope is the basic unit that drives antigen function and is closely related to the immune response.[22-23] Immunodominant epitopes are particularly important in vaccine development.[24] Adverse reactions and immune escape after vaccination are closely associated with immunodominant epitopes. However, the current understanding of epitopes, their composition, and recombination remains unclear. Therefore, this study focuses on these issues.

We previously reported that three strains of mAbs against the influenza virus HA protein were located on the same peptide, that is, H1-5, H1-16, and H1-81 bound to WSYIVE (93aa-98aa), and A1-10, H1-74, and H1-84 bound to LVLWGIHHP (191aa-199aa) (Figure S1). The variability in the amino acid sequences of the variable regions among these antibodies suggests that they are not derived from the same hybridoma cells (Figure S2A and B). The unique reaction characteristics exhibited by the three mAbs binding to the same peptide (Table 1) imply that the epitopes to which these three mAbs bind may not be identical, indicating that a 6-peptide can constitute multiple antigen epitopes. Analysis of antibody-binding epitopes by alanine scanning revealed that 1–2 amino acid differences between the immunodominant groups bound by each mAb (Figure 1), which may explain why the three mAbs show different reactions. The production of these mAbs may be activated by a combination of different immunodominant groups on the peptides. The researchers used alanine

scanning replacement method to study the peptide and found that the immunodominant group played a key role.[25,26] These results indicated that the epitope may be a combination of immunodominant groups, such that a single peptide may form several epitopes.

Previous studies have confirmed the minimum number of B cell epitopes at 5–6 amino acid residues. The physical properties of many amino acid residues are important for epitope formation.[27] We found that a short peptide could form multiple epitopes. Therefore, mAbs were prepared using LVLWGIHHP and WSYIVE of the influenza virus HA protein as immunogens. The mAbs generated for the two peptides displayed diverse reactions toward influenza virus antigens (Table 2 and Table S2). Table 2 illustrates the response characteristics of seven mAbs derived from WSYIVE that target different subtypes of influenza virus antigens. Based on their reactivity, the epitopes developed by WSYIVE can be categorized into two or more types that are either identical or dissimilar to the influenza virus antigens. Thus, multiple epitopes may be present in a single peptide.

The interaction between the peptide and antibody relies on surface accessibility and specific groups in the polypeptide (Figure 2). The chemical groups in a peptide with an immunodominant role can bind to different antibodies in various combinations.[28] As shown in Table S4B, when the peptide WSYIVE was bound to H1-5, the involvement of serine (S) and glutamic acid (E) was more pronounced than that of tyrosine (Y) and valine (V) when binding to H1-16. However, they had a more significant role in binding to other mAbs. Similarly, as shown in Table S4A, glycine (G) located on the peptide was more crucial for binding with H1-74 and H1-84, as evidenced by the higher binding energies. The combination of histidine (H) and proline (P) exhibited a lower impact on binding to H1-74 than with A1-10 and H1-84. We concluded that the immunodominant groups present in the peptide play different roles in the binding process with different antibodies, thus providing a theoretical basis for designing recombinant multiple-epitope peptides.

In recent years, computer-aided prediction has yielded fruitful results in studying antigen epitopes.[29,30] Guevarra used computer analysis tools to predict the specific

immunogenic epitope of the DENV type 2 NS1 antigen, synthesized simulated peptides to immunize rabbits and found that rabbits produced antibodies that could specifically bind the simulated peptides.[31] Javadi used computer models and immune informatics tools to design effective multi-epitopes of microbial antigens, particularly the aggregation of immunogenic epitopes of multiple antigens, to produce highly immunogenic multi-epitope recombinant antigens. These results are of significance for disease prevention and the development of diagnostic reagents.[32] Several methods and online tools for modifying and designing antigen epitopes have been established from various perspectives based on computer simulations.[33-35] Using these tools, scientists have combined computer prediction with laboratory validation to study epitopes related to microorganisms, such as the Zika virus, chikungunya virus, Hantavirus, and Toxoplasma gondii.[36-38] However, these results are based on the amino acid sequence of the antigen, thereby limiting their further development. For example, during the transmission of the novel coronavirus, amino acids in key regions of the virus continue to mutate which affects the immune efficacy of the vaccine.[39,40] Based on the theory that epitopes are immunodominant groups, it is possible to recombine multi-epitope peptides with better immunogenicity.

In this study, 61 multi-epitope peptides were synthesized by computer molecular simulations utilizing the merging and recombination of immunodominant groups linked by six mAbs (Figure S3). We observed that six recombinant multi-epitope peptides exhibited good binding activity with the six mAbs (all with a binding force greater than 8.2 Kcal/mol) by modeling the structural homology of the variable region proteins of the six mAbs (Table S3) and molecular simulation of binding to 61 peptides (Table 3). Despite the dissimilarity between the recombinant multi-epitope peptide sequences and the two peptides, LVLWGIHHP and WSYIVE, XH-001 and XH-006 exhibited a significant inhibition rate of more than 0.8 against the binding of each mAb to the HA antigen of the influenza virus (Figure 3A).

Further identification showed that the epitopes binding to the six mAbs were well preserved on the recombinant multi-epitope peptide, XH-001 (Figure 3B and 3C), whereas the epitopes binding to the mAbs H1-74, H1-84, H1-5, and H1-81 of XH-006

were lost. This may be because the labeling with BSA resulted in changes in the structure of XH-006. Therefore, XH-001 (ILLWVLSHL) was selected for further functional analyses. Antiserum was obtained by immunizing mice with the XH001-BSA peptide and purified. The antigenic epitopes of the LVLWGIHHP, WSYIVE, and HA proteins could be successfully transferred to the recombinant multi-epitope peptide XH-001 (Table 4). The protective effects of XH-001 were examined at the cellular level, as shown in Figure 4. The protective effect of the recombinant multi-epitope peptide was greater than that of a single epitope, indicating that the method of carrying multiple epitopes with a single peptide established in this study is feasible. Compared with baculovirus as cell gene delivery vector to construct multi-antigen chimeric epitope vaccine,[41] the method established in this study was easier to promote and apply, and can also eliminate cross-reactive epitopes with human tissues to avoid adverse reactions. At present, the immunogenicity of peptides is not as good as that of macromolecular antigens. Our team previously demonstrated that the binding force of antigen epitope and antibody can be improved by computer virtual mutated immunodominant groups, [42] which provides a reference for us to further improve the immunogenicity of recombinant peptides.

In this study, a new method of recombinant multi-epitope peptide antigen using the variable region of mAb as counterpart was established by computer molecular simulation technique. It provides new ideas for polyvalent vaccines, new diagnostic reagents related to peptides and research and development of peptide drugs. Future，we will prepare a large number of mAbs against different respiratory pathogens, correspond the antibody to the immunodominant group of the antigen, establish a database, and then recombine the universal multi-epitope peptide antigen for a variety of respiratory pathogens, so as to provide a research basis for the prevention and control of infectious diseases. In addition, the method can also be used in the discovery of tumor-associated antigens, tumor vaccine development and autoimmune disease research.

Figure 1. mAbs binding to the same peptide recognize different immunodominant groups. (A) Immunodominant groups of H1-5, H1-16, and H1-81 mAbs recognize the WE-6. (B) Immunodominant groups of A1-10, H1-74, and H1-84 mAbs recognize the LP-9. (C) Differences in the combination of immunodominant groups to which the six mAbs bind.

Figure 2. Binding interactions between mAbs and peptides.
Amino acids within the range of 0.4 nm between mAbs and peptides were regarded as key residues forming the binding interface. mAbs and peptides are shown in light green and blue, respectively. Polar hydrogen bonds between the peptides and antibodies are indicated by dashed lines. Binding energies of LP-9/A1-10 (A), LP-9/H1-74 (B), LP-9/H1-84 (C), WE-6/H1-5 (D), WE-6/H1-16 (E) and WE-6/H1-81 (F) were -20.7 kcal/mol, -21.9 kcal/mol, -22.8 kcal/mol, -14.63 kcal/mol, -15.98 kcal/mol and -15.40 kcal/mol, respectively.

Figure 3. Identification and analysis of six mAbs against the HA antigen that recognizes recombinant multi-epitope peptides. (A) Competitive ELISA for the reactivity of the identified recombinant multi-epitope peptides with the six mAbs. The H1N1 influenza virus HA (5 μg/mL) was used as a coating antigen. The recombinant multi-epitope peptides (100 μg/mL) were used for inducing the competitive binding of HA with six mAbs (3 μg/mL). (B and C) Indirect ELISA of the reactivity of recombinant multi-epitope peptides with six mAbs. (B) The peptides XH001-BSA, XH006-BSA, and H1N1 influenza virus HA and a control peptide (10 μg/mL) were used as coating antigens. Six mAbs (3 μg/mL) was used to detect the coated peptides. (C) Peptide XH-001 was diluted 10-fold and used to coat 96-well plates. Six mAbs (3 μg/mL) were used to detect the coated peptides.

Figure 4. Neutralizing activity of the XH-001 immunized serum. (A-D) The neutralization activity of LP-9 antibody, WE-6 antibody, XH-001 immunized serum, and control antibody against A/California/07/2009(H1N1) were evaluated, respectively. Two-fold serial dilutions of antibodies (initial concentration of 3.0 mg/mL) and control antibodies were prepared. (E) MDCK cells in the neutralization activity of XH-001 immunized serum. 1: Blank control, DMEM was added instead of A/California/07/2009(H1N1) and XH-001 immunized serum. 2: XH-001 immunized serum and A/California/07/2009(H1N1) was used to test the protective effect. 3: Control antibody (SP2/0 control antibody) and A/California/07/2009(H1N1) was used as experiment control.

AUTHOR CONTRIBUTION STATEMENT

Jun Hu designed the study. Chunyan Guo，Cuixiang Xu and Lijun Shang wrote the manuscript. Qing Feng，Xin Xie，Yan Li performed the experiments. Senbiao Fang guided computer simulations. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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